SUPPLEMENTAL MATERIALS:

Figure S1. (A) Dose-dependent interaction of Rabin8(262-331) to Sec15. Various amounts of in vitro translated [^{35}S] labeled Sec15 were incubated with GST-tagged Rabin8 fragment containing a.a. 262-331 in the binding experiment. "Input" represents 0.5 μl of in vitro translated [^{35}S] labeled Sec15. **(B)** The binding curve showing various amounts of Sec15 bound to Rabin8(262-331). The amount of Sec15 binding was standardized to 1 μl in vitro translated [^{35}S]-labeled Sec15 (defined as 1 A.U.). Error bars, standard deviation (n=3). **(C)** Binding of Rabin8(262-331) to Rab11[Q70L]. GST and GST-tagged Rabin8, Rabin8(Δ300-305), and Rabin8(262-331) were incubated with Nus-Hisx6-tagged Rab11[Q70L] fusion protein in the binding assay. The full-length Rabin8 bound to Rab11[Q70L] whereas Rabin8(Δ300-305) had a much weaker interaction with Rab11[Q70L]. The Rabin8 fragment containing a.a.262-331 had no detectable binding to Rab11[Q70L].

Figure S2. GFP-Rabin8 co-localizes with the Rab11[Q70L] in HeLa cells, whereas GFP-Rabin8Δ(300-305) is diffused in the cytoplasm. Scale bar, 10 μm.

Figure S3. Cells expressing Rabin8Δ(300-305) have slightly shorter cilia length. **(A)** hTERT-RPE1 cells were transfected with either GFP-Rabin8 or GFP-Rabin8Δ(300-305). The length of primary cilia in these cells was determined by immunostaining of acetylated α-tubulin (red). GFP-Rabin8Δ(300-305)-expressing cells had slightly shorter cilia than cells expressing GFP-Rabin8. Nuclei were stained with DAPI (blue) and the merged images are shown to the right. Scale bar, 10 μm. (**B**) Quantification of cilia length in cells expressing GFP-Rabin8 or GFP-Rabin8Δ(300-305). The data were analyzed using Student's t-test and presented as means with standard error (n=60, $p<0.01$).

Figure S4. Overexpression of Sec15 arrests the recycling of endocytosed transferrin. hTERT-RPE1 cells were transfected with GFP or GFP-Sec15. After incubated with Texas Red-labeled transferrin (40 μg/ml) for 5 min at 37°C, the cells were changed to the DMEM/F12 media containing 10% fetal bovine serum for different times. The cells were placed on ice to terminate trafficking and fixed with 4% PFA. At 0 min time after changing to the DMEM/F12 media, transferrin-contained vesicles were distributed throughout the cytoplasm, reflecting transport to early endosomes (left panel). After 45 min, in GFP-expressing cells, the transferrin fluorescence signal was significantly reduced due to their release from the cells. However, in cells overexpressing GFP-Sec15, much of the transferrin remained co-localized with GFP-Sec15 at the perinuclear recycling endosome region.

Figure S1.

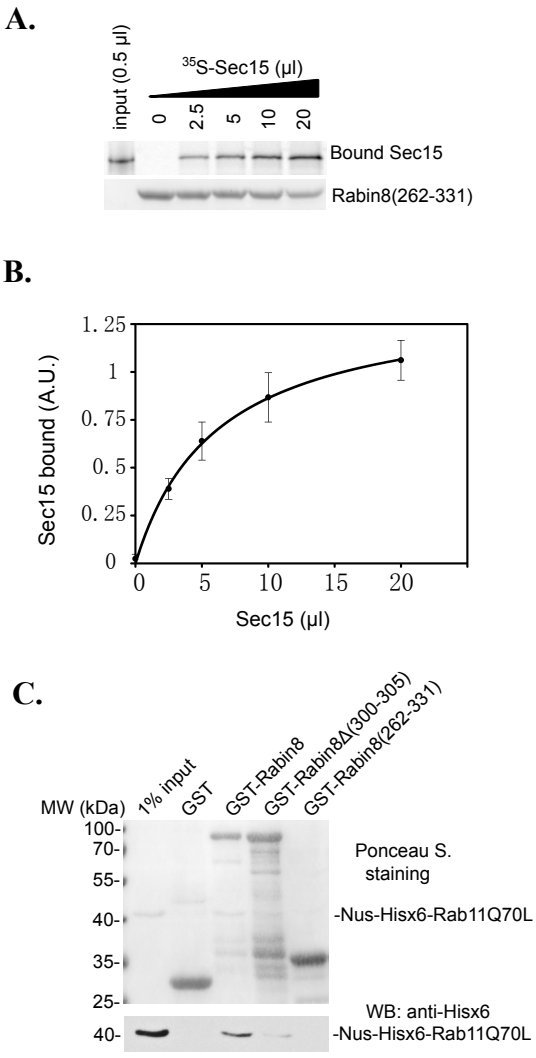

A.
input (0.5 µl)
35S-Sec15 (µl)
0
2.5
5
10
20
Bound Sec15
Rabin8(262-331)
B.
Sec15 bound (A.U.)
1. 25
1
0. 75
0. 5
0. 25
0
0
5
10
15
20
Sec15 (µl)
C.
MW (kDa)
1% input
GST
GST-Rabin8
GST-Rabin8Δ(300-305)
GST-Rabin8(262-331)
100-
70-
55-
40-
35-
25-
40-
Ponceau S. staining
-Nus-Hisx6-Rab11Q70L
WB: anti-Hisx6
-Nus-Hisx6-Rab11Q70L

Figure S2.

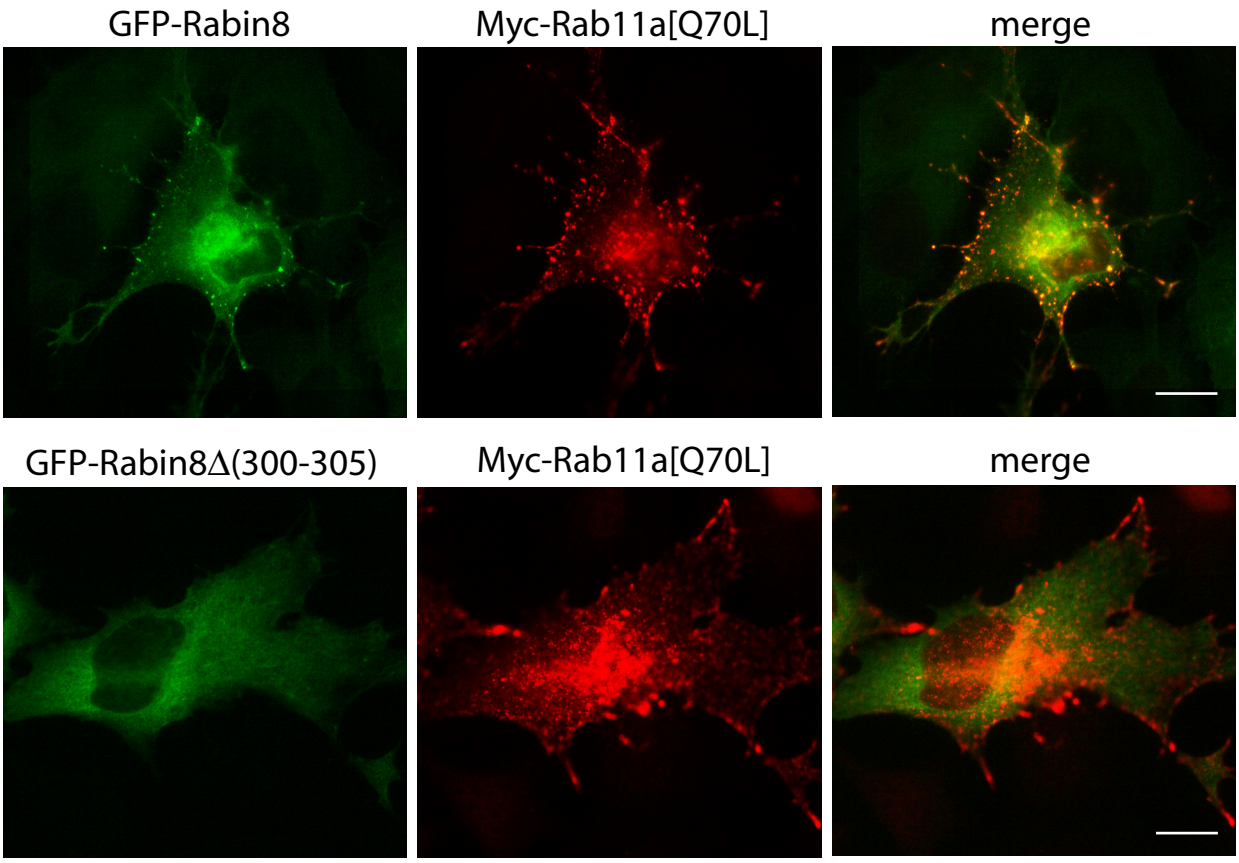

GFP-Rabin8
Myc-Rab11a[Q70L]
merge
GFP-Rabin8Δ(300-305)
Myc-Rab11a[Q70L]
merge

Figure S3.

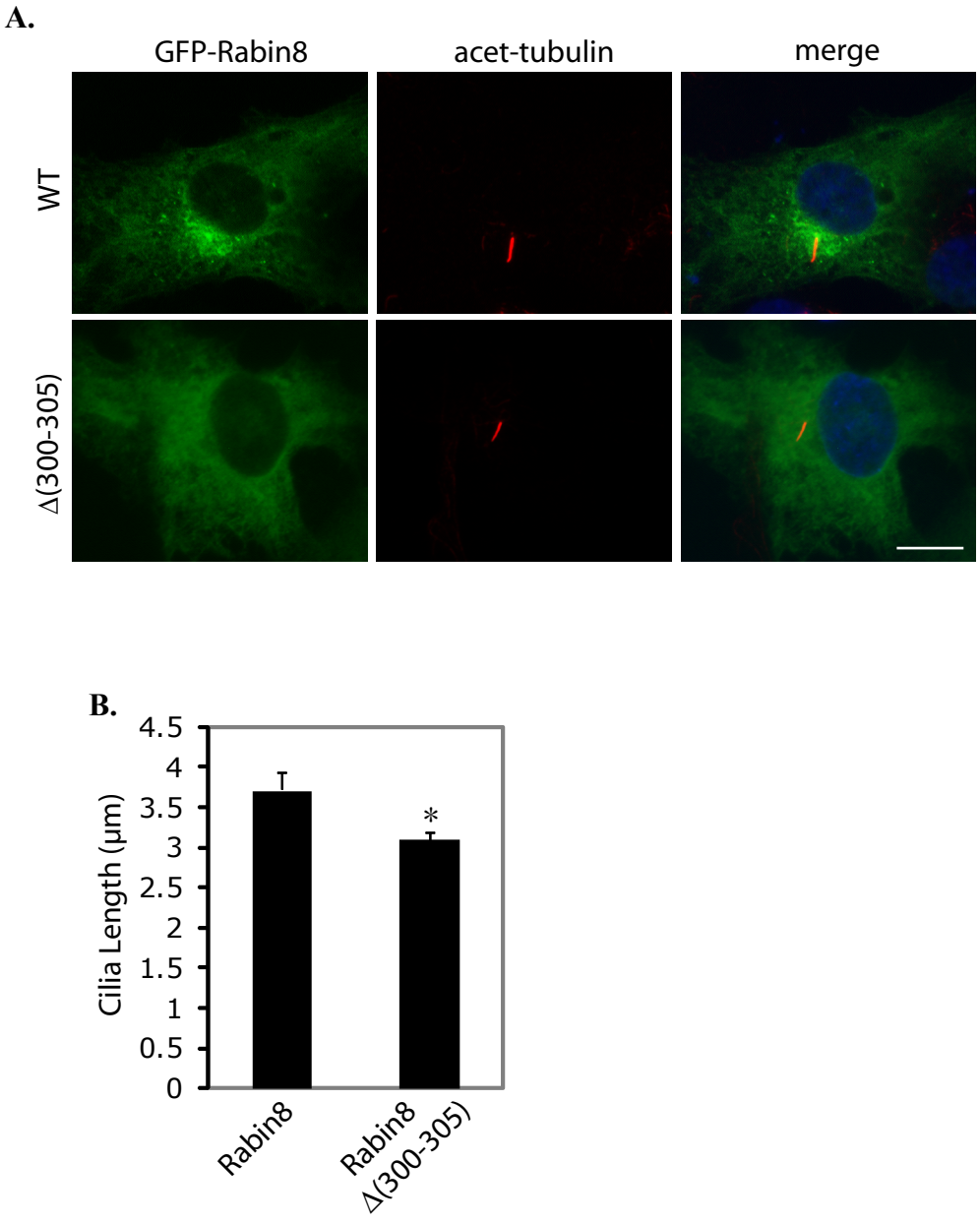
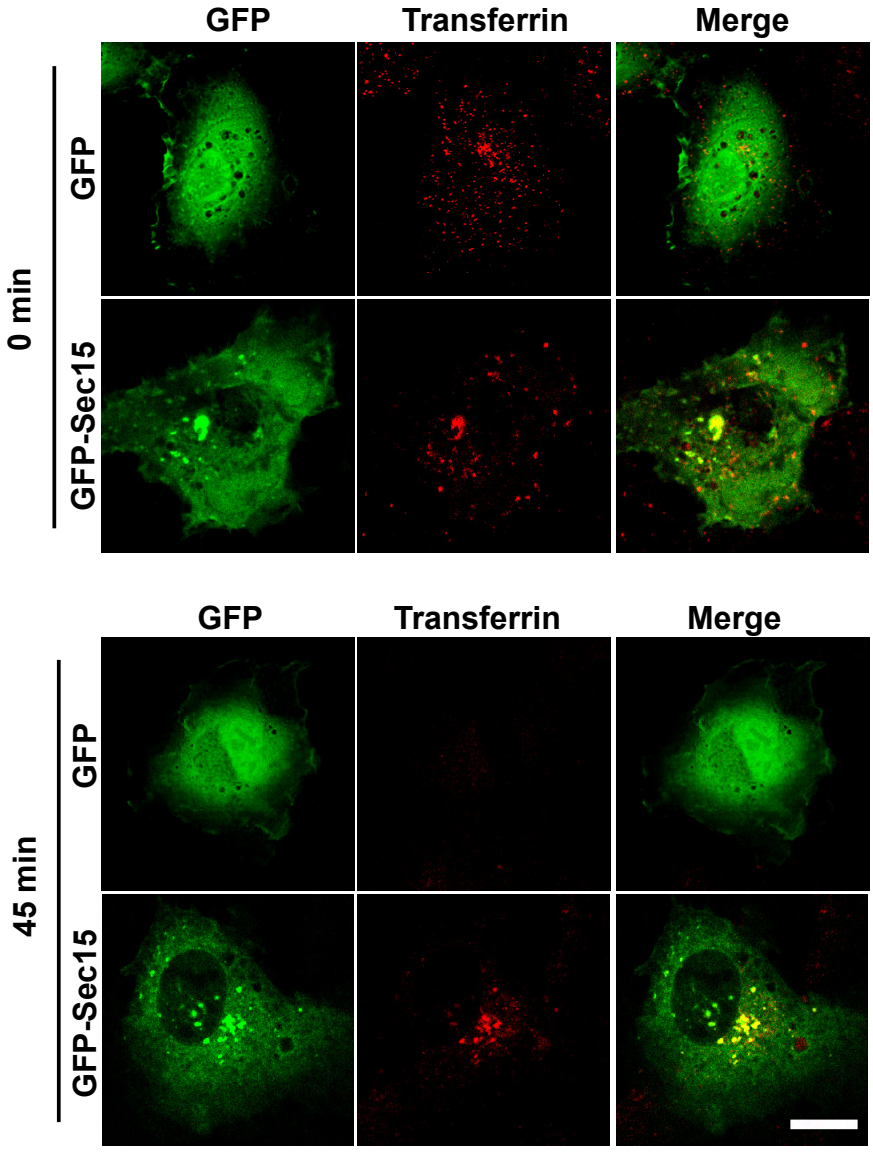

A.
GFP-Rabin8
acet-tubulin
merge
WT
Δ(300-305)
B.
Cilia Length (μm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
Rabin8
Rabin8 Δ(300-305)

Figure S4.


GFP
Transferrin
Merge
0 min
GFP
GFP-Sec15
GFP
Transferrin
Merge
45 min
GFP
GFP-Sec15